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Serum Isoenzyme Pattern of Creatine Kinase and Lactate Dehydrogenase in Various Animal Species

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Summary: The creatine kinase and lactate dehydrogenase isoenzyme pattern were determined in the serum of normal and untreated rats, rabbits, dogs, monkeys and pigs. The relative distribution of all isoenzymes in the serum and an electrophoretic pattern for each animal species are presented. The isoenzyme serum pattern showed a great variation between the species. The diagnostic value of serum creatine kinase isoenzyme MB and lactate dehydrogenase isoenzymes 1 and 2 in predicting cardiac lesions in different animal species is briefly discussed.

Introduction

The determinations of creatine kinase[1]) and lactate dehydrogenase[1]) isoenzymes in the serum have been quite useful in the diagnosis of specific tissue damage in heart, skeletal muscle and liver disorders in humans. However, these methods have not been so fully explored in a variety of animal species, specially in toxicological studies.

Recently we have shown that the determinations of creatine kinase isoenzyme MB and lactate dehydrogenase isoenzymes 1 and 2 in the serum are valuable indicators of early cardiac damage in rats after isoproterenol application (1). There are fewer reports in the literature concerning creatine kinase and lactate dehydrogenase isoenzymes in the serum of different animal species. Moreover, the methods employed differ with respect to the carrier used for electrophoretic separation, or the substrate used for staining, resulting in different detection limits for these isoenzymes (2–8).

Therefore, it was of interest to examine the normal serum isoenzyme pattern of creatine kinase and lactate dehydrogenase in various animal species commonly employed for pharmacological and toxicological studies, using improved methods under standardized conditions.

Materials and Methods

Six male animals per species were employed in this study. The strain and the body weight of the animals were as follows: Wistar-Han rats (240–260 g), New Zealand white rabbits (2.1–2.3 kg), Beagle dogs (10.5–11.8 kg) and *Macaca fascicularis* monkeys (3.3–3.9 kg). Furthermore, six castrated male pigs of the German Landrace (30–35 kg) were also employed.

The animals were housed in individual cages under conventional conditions and were fasted 17–20 h prior to blood collection. The blood collection for the serum samples was performed without anaesthesia from the v. jugularis in the case of rats, rabbits and dogs and from the v. antebrachia in monkeys. In pigs, the blood was collected via a catheter inserted into the a. carotis dextra under i. v. nembutal anaesthesia.

Determinations of total creatine kinase and total lactate dehydrogenase serum activities (catalytic concentration in U/l) were performed according to optimized methods as recommended by German Society for Clinical Chemistry at 25 °C using test combinations from Boehringer Mannheim, West Germany, with the automatic analyser Hitachi 705.

Determinations of creatine kinase and lactate dehydrogenase isoenzymes were performed using test combinations from Corning Medicals, Palo Alto, CA (article nos. 470010090 and 470010100). These determinations were carried out essentially as prescribed by Corning Medicals. Creatine kinase and lactate dehydrogenase isoenzymes were separated on agarose films using a Corning electrophoresis chamber. Separation conditions

[1]) Enzymes
Creatine kinase (EC 2.7.3.2),
Lactate dehydrogenase (EC 1.1.1.27)

for creatine kinase isoenzymes: MOPSO [3-(N-morpholino)-2-hydroxypropane sulphonic acid] buffer, pH 7.8, and 20 minutes separation time; for lactate dehydrogenase isoenzymes: universal barbital buffer, pH 8.6 and 35 minutes separation time. Creatine kinase isoenzyme fractions were quantified by scanning the fluorescence on the dried film at excitation and emission wavelengths of 366 and 460 nm respectively, with a chromatographic spectrophotometer model KM3, Zeiss, West Germany. Lactate dehydrogenase isoenzymes were quantified after substrate staining with nitro blue tetrazolium (NBT) and scanning the film at 600 nm with a densitometer, model CDS-100, Beckman, West Germany. The relative distribution of the isoenzymes are expressed in percent of the total creatine kinase and lactate dehydrogenase activity. The detection limit for each isoenzyme in serum was about 2–5 U/l as stated by Corning Medicals, Palo Alto, using 1–2 µl of serum samples. The specificity of the isoenzyme bands as well as the accuracy of the methods were controlled by using creatine kinase and lactate dehydrogenase isoenzymes quality control serum of Gilford, Ciba Corning diagnostic Corp. (Lot No. 051603).

The determinations of total creatine kinase and lactate dehydrogenase activity as well as their isoenzymes were performed on the same day as the blood collection in rats, rabbits, dogs and monkeys. In pigs, all blood samples were collected over a period of 3 weeks for technical reasons. The determinations of total creatine kinase and lactate dehydrogenase activity in pig serum were performed on the same day as the blood collection. Thereafter, the samples were frozen at −20 °C prior to determination of creatine kinase and lactate dehydrogenase isoenzymes, since the relative distribution of these isoenzymes was found to be stable over a period of 4 weeks at −20 °C.

Results and Discussion

A typical serum creatine kinase isoenzyme pattern of each animal species is illustrated in figure 1.

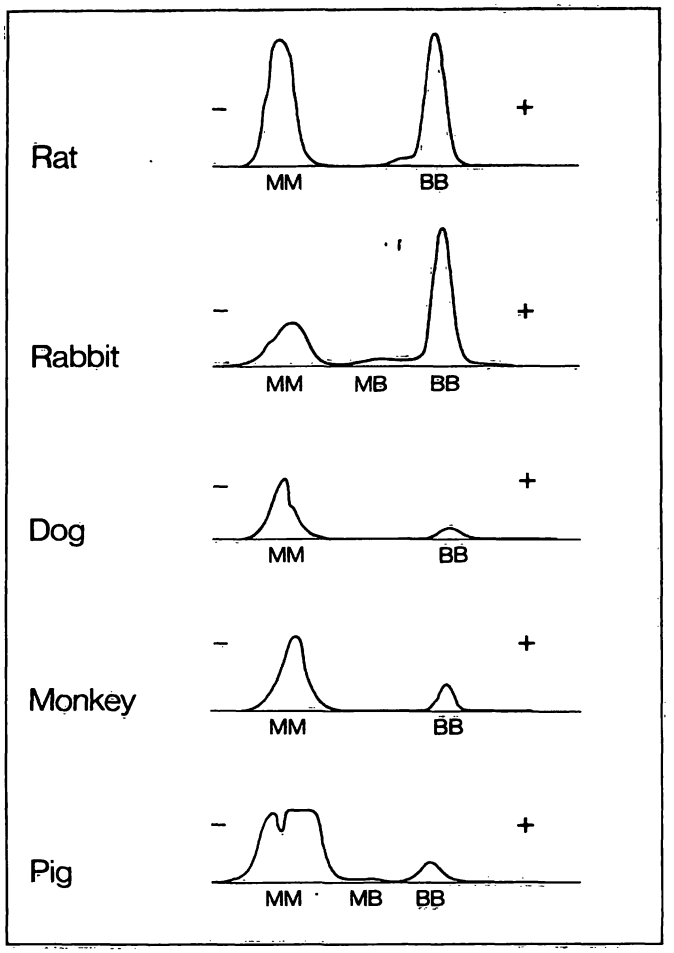

Fig. 1. Typical creatine kinase isoenzyme pattern in the serum of various untreated animal species.

The total creatine kinase activity and the relative distribution of the creatine kinase isoenzyme activities in the serum of normal rats, rabbits, dogs, monkeys and pigs are presented in table 1.

Tab. 1. Total creatine kinase and creatine kinase isoenzyme activities in the serum of various untreated animal species ($\bar{x} \pm s$, range; n = 6)

Animal species	Total creatine kinase [U/l]	Relative distribution of creatine kinase isoenzymes [%]		
		MM	MB	BB
Rat	172 ± 70 (80–260)	61 ± 10 (48–71)	–	39 ± 10 (29–52)
Rabbit	402 ± 135 (204–614)	42 ± 17 (21–63)	8 ± 7 (0–17)	51 ± 19 (28–75)
Dog	67 ± 36 (41–127)	86 ± 4 (80–91)	–	14 ± 4 (9–20)
Monkey	113 ± 127 (39–365)	80 ± 8 (73–95)	–	20 ± 8 (5–27)
Pig	865 ± 220 (634–1148)	89 ± 3 (83–92)	2 ± 1 (1–3)	9 ± 3 (7–15)

The creatine kinase isoenzyme MM (muscle type) was the predominant fraction in the serum of rats, dogs, monkeys and pigs, whereas in the case of rabbits, the creatine kinase isoenzyme BB (brain type) was mainly found in the serum. No activity of the creatine kinase isoenzyme MB (heart type) was detected in the serum of rats, dogs and monkeys and only small amounts of this isoenzyme were present in the serum of rabbits and pigs (see table 1).

It is known from the literature that in the heart muscle 30–41% of the total creatine kinase activity occurs as MB isoenzyme in humans, 28 ± 2% in rats, 13 ± 4% in dogs and 4% in pigs (2, 8, 9, 10). A rise in MB isoenzyme activity in the serum has been reported in rats after isoproterenol-induced cardiac damage (1), in dogs after occlusion of the left anterior descending coronary artery (9) and in rabbits in blood samples obtained after cardiac puncture (4). These observations demonstrate that the measurement of MB isoenzyme activity in the serum could be useful for the diagnosis of cardiac lesions in these species.

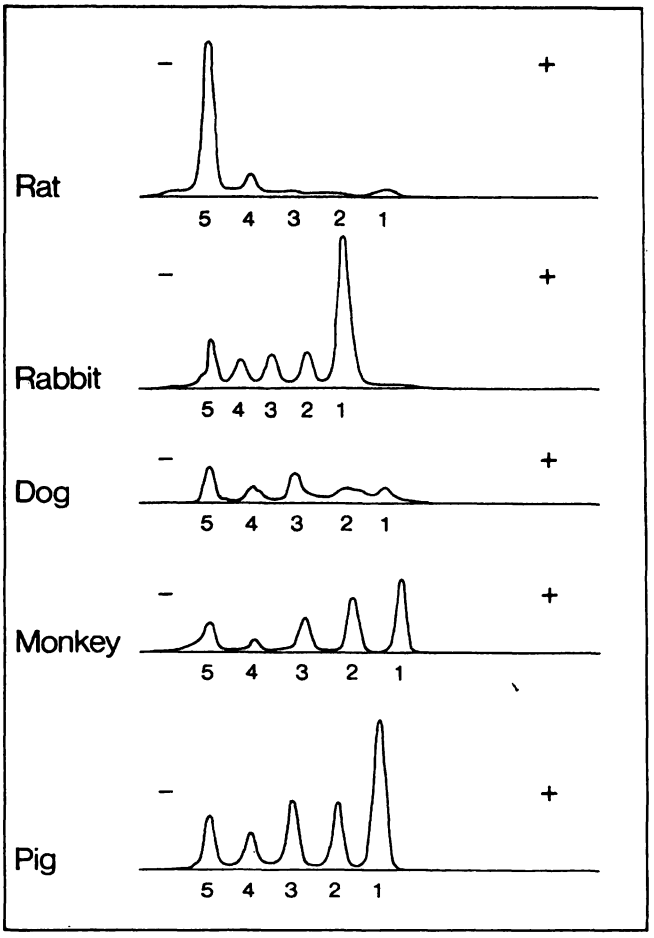

Fig. 2. Typical lactate dehydrogenase isoenzyme pattern in the serum of various untreated animal species.

This could also apply to monkeys as it is known that the heart muscle of this species also contains a high fraction of MB isoenzyme activity (11).

Only a very low amount of creatine kinase isoenzyme MB occurs in pig myocardium and no rise in the serum activity of this isoenzyme was observed in pigs with severe myocardial damage after infusion of succinylcholine-chloride (8). Thus, the determination of MB isoenzyme activity in the serum seems to be of no great value for the diagnosis of cardiac damage in this species.

A typical serum lactate dehydrogenase isoenzyme pattern of each animal species is illustrated in figure 2.

The total lactate dehydrogenase activity and the relative distribution of the lactate dehydrogenase isoenzyme activities in the serum of normal rats, rabbits, dogs, monkeys and pigs are presented in table 2.

Lactate dehydrogenase isoenzyme 5 was the predominant fraction in rat serum and only low proportions of the lactate dehydrogenase isoenzymes 1−4 were found. Rabbit and pig serum mainly revealed the presence of lactate dehydrogenase isoenzyme 1 and fairly similar proportions of the isoenzymes 2−5. In dog serum, the lactate dehydrogenase isoenzyme 3 was the major fraction followed by the isoenzymes 2, 5, 4 and 1. The serum lactate dehydrogenase isoenzyme pattern of monkeys mainly consisted of the isoenzymes 1−3 and 5 followed by isoenzyme 4 (see tab. 2).

High proportions of lactate dehydrogenase isoenzymes 1 and 2 activity are known to occur in the myocardium, and also in the kidneys and brain in all animal species employed in the present study (8, 11−17). Furthermore, as is known from the literature, the erythrocytes of monkeys and pigs also contain a high percentage of the lactate dehydrogenase isoenzymes 1 and 2, whereas those of rats contain only lactate dehydrogenase isoenzyme 5 (18, 19).

Tab. 2. Total lactate dehydrogenase and lactate dehydrogenase isoenzyme activities in the serum of various untreated animal species ($\bar{x} \pm s$, range; $n = 6$)

Animal species	Total lactate dehydrogenase [U/l]	Relative distribution of lactate dehydrogenase isoenzymes [%]				
		Isoenzyme 1	Isoenzyme 2	Isoenzyme 3	Isoenzyme 4	Isoenzyme 5
Rat	239 ± 132 (128−467)	6 ± 2 (3−8)	3 ± 1 (1−5)	4 ± 2 (2−6)	10 ± 4 (3−13)	78 ± 8 (71−89)
Rabbit	231 ± 76 (162−361)	50 ± 9 (37−60)	13 ± 2 (9−15)	13 ± 2 (11−16)	10 ± 3 (6−13)	15 ± 6 (8−24)
Dog	54 ± 22 (38−98)	11 ± 3 (6−16)	23 ± 4 (17−28)	30 ± 4 (26−36)	14 ± 2 (13−18)	22 ± 3 (17−26)
Monkey	225 ± 28 (184−257)	28 ± 4 (22−32)	25 ± 3 (20−29)	22 ± 5 (17−27)	9 ± 2 (6−11)	17 ± 8 (9−30)
Pig	553 ± 60 (485−627)	37 ± 3 (33−42)	16 ± 1 (14−17)	18 ± 1 (17−20)	12 ± 2 (9−16)	17 ± 3 (14−20)

An increase in the serum activities of lactate dehydrogenase isoenzymes 1 and 2 was demonstrated in the case of cardiac damage after isoproterenol application in rats (1, 3) as well as after succinylcholine-chloride infusion in pigs (8). The measurements of lactate dehydrogenase isoenzymes 1 and 2 in non-haemolytic serum samples may also be valuable indicators of cardiac damage. However, in this case, the kidneys should be excluded as a source of lactate dehydrogenase isoenzmyes 1 and 2, on the basis of either serum urea nitrogen and serum creatinine or urinary enzyme determinations.

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